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PRIMARY RESEARCH PAPER

Bacterial abundance and aerobic microbial activity across natural and oyster aquaculture habitats during summer conditions in a northeastern Pacific estuary

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Abstract We measured sediment properties and the abundance and aerobic metabolism of microbes in Willapa Bay, Washington, USA, to test the response of sediment microbes to oyster aquaculture. Sites spanned the estuary gradient (practical salinity units ranged from 24 to 30 under seasonally low river flows) and six different low-intertidal habitat types: eelgrass (*Zostera marina*), unstructured tideflat, oyster hummocks (reefs of *Crassostrea gigas*), longline oyster aquaculture, hand-picked on-bottom oyster aquaculture, and dredged on-bottom oyster aquaculture. Aerobic metabolism was assessed by sole-source carbon use (SSCU) of 31 carbon sources on Biolog plates. Sediments generally became siltier and more organically enriched into the estuary, but no consistent differences in sediment properties occurred across habitat types. Bacterial cell density tracked organic content. Across the estuary gradient, overall aerobic SSCU increased less steeply than bacterial cell density, possibly as anaerobic metabolism became more important. Across habitats, aerobic SSCU differed significantly in both overall metabolism and diversity of carbon sources. Aerobic metabolism was generally lower for sediment microbes from intertidal on-bottom oyster aquaculture than from eelgrass. Humans indirectly alter microbial activity through biogenic habitats created during aquaculture, but, as has been shown for bivalves more generally, these changes were relatively small, particularly in comparison to sediment changes along estuarine gradients.

Keywords Aquaculture · Bacteria · *Crassostrea gigas* · Microbiological analysis · Prokaryotes · Sediments · *Zostera marina*

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Introduction

Quantitative assessment of estuarine microbial assemblages in relation to environmental determinants provides important clues to ecosystem function in these highly productive habitats. Although microbes are ubiquitous, they show fine-scale variation in community structure in response to environmental variability in space and time (Horner-Devine et al., 2004). The ways in which microbes respond to natural and anthropogenic change in estuaries are not entirely clear (Paerl et al., 2002), but the consequences may be substantial due to concomitant alteration of the biogeochemical cycles they support. Sediment microbes are influenced by a variety of estuarine pollutants, including nutrients (Meyer-Reil & Koster, 2000), heavy metals (Dell'Anno et al., 2003), and hydrocarbons (Yakimov et al., 2005). Certain types of aquaculture have similar effects. Microbial abundance tends to increase in sediments beneath fish farms (Rajendran et al., 1999; Caruso et al., 2003), and this may be accompanied by a shift in community composition and lower per-cell activity (Vezzulli et al., 2002). In contrast, few studies have examined microbial response to bivalve aquaculture; existing cases indicate small effects despite high densities of macrofauna (Rajendran et al., 1999; Danovaro et al., 2004). Here, we focus on the relative influences of natural and human-induced environmental factors on microbial assemblages in Willapa Bay, Washington, USA, where long-term management of these tideflats for oyster culture provides an opportunity for comparisons of microbial assemblage response to natural environmental gradients and several oyster culture practices.

In previous studies, several abiotic factors have been shown to correlate with microbial abundance, community structure, and community function in estuarine sediments. Three major influences are location along the estuarine gradient, sediment grain size, and depth in the sediment. Methanogens dominate prokaryotic communities in fresher locations, whereas sulfate-reducing bacteria are more common under marine influence (Purdy et al. 2002a, b), consistent with the availability of chemical substrates for metabolism. Microbial densities and activity generally increase with the presence of fine-grained particles in sediment (Blum et al., 2004; Koster et al., 2005). Deeper in sediments, densities of microbes tend to decline (Tankere et al., 2002), and functional types shift from aerobic to anaerobic, except when sediments are vertically mixed (Riese, 1985; Koretsky et al., 2005).

In estuaries, macrobiota also influence microbial assemblages, either by directly affecting microbes or by modifying local abiotic conditions (Fry, 1982). Direct effects occur as macrobiota consume microbes, for instance, deposit feeders can alter species composition during their feeding activities (Wilde & Plante, 2002). Indirect effects on microbes have been documented for rooted macrophytes, which create aerobic conditions around their roots, in addition to adding organic material (O'Donohue et al., 1991; Welsh et al., 1996; Donnelly & Herbert, 1999; Cifuentes et al., 2000). Burrowing animals aerate and bioturbate sediments, thereby changing local conditions for microbes (Riese, 1985), whereas suspension-feeding animals add fecal material and reduce sediment grain size, often creating anoxic conditions (Fry, 1982; Deslous-Paoli et al., 1992). This sediment change may be particularly dramatic when bivalves are at high density in aquaculture (Kaspar et al., 1985; Kautsky & Evans, 1987; Hatcher et al., 1994). Indeed, biodeposits from filter feeders can alter nutrient flux rates, presumably through the intermediate step of microbial activity (Newell et al., 2002).

In this study, we explore the effect of oyster aquaculture on microbes in Willapa Bay, Washington. In particular, we compare the effect of different oyster (introduced, *Crassostrea gigas* Thunberg) aquaculture techniques on cell densities and aerobic metabolism by sediment microbes. Willapa Bay has the largest commercial oyster production of any estuary in the US (Ruesink et al., 2006). A variety of techniques are used to grow oysters in Willapa Bay: mechanical dredge harvest of on-bottom oysters, hand harvest of on-bottom oysters, and longline aquaculture in which oysters are strung on ropes between stakes about 0.5 m above the sediment. Substantial variability also exists in the frequency of harvest operations: "fattening" beds may have oysters moved on and off within a few months, whereas "seed" or "grow out" beds may hold oysters for several years. In addition, *C. gigas* recruits naturally in some areas, forming consolidated shell habitats ("hummocks") of 1–100 m^2, which are allowed to develop and then picked for oysters in some areas of the bay. Natural habitats in the bay's low-intertidal

zone include eelgrass beds (*Zostera marina* L.) and unstructured mud/sand. The latter contain no sessile macroscopic organisms above the surface, but infauna are common, for instance, burrowing thalassinid shrimp, or clams and worms (Ferraro & Cole, 2004).

We collected sediment and microbe samples from Willapa Bay during the most productive period for benthic organisms: eelgrass has its peak turnover and therefore detrital production at mid-summer (Ruesink et al., 2006), and oysters have seasonally high biodeposition due to temperature-dependent filtration (Ren & Ross, 2001). This period may represent the least favorable conditions for aerobic metabolism due to additions of labile organic material, however it is likely to show the most extreme differences among habitats with varying amounts of influence from benthic macrobiota.

Shifts in microbial communities would be expected in oyster habitats relative to unstructured tideflat or eelgrass, given strong responses of microbes to macrobiota (Fry, 1982), but whether such shifts occur in response to shellfish aquaculture has not been studied. We examined microbial communities across three sites, each consisting of six habitat types, including shellfish aquaculture, in Willapa Bay, Washington. Collections from additional sites were included to assess variation across the estuarine gradient. The tideflats of this northeastern Pacific estuary have sustained little human modification except for local areas of intensive shellfish aquaculture (Ruesink et al., 2006). Our analysis of microbes is one part of a larger study to understand ecosystem-level effects of shellfish aquaculture, particularly on the biogeochemistry of the sediment and water column.

Materials and methods

Study sites

Willapa Bay (46°40′ N, 124°0′ W) is a coastal macrotidal estuary, connected to the Pacific Ocean by a shallow entrance about 10 km across in the northwest corner. More than half of the bay's ~35,000 ha (350 km^2) is exposed during extreme low tides (Borde et al., 2003). We have proposed that the two arms of Willapa Bay function differently in terms of energetic support of food webs (Ruesink et al., 2003). The eastern arm is a typical estuary with high watershed inputs from two large rivers. The southern arm, which is fed by smaller rivers, is a coastal lagoon with declining productivity away from the ocean. River flow in general is low during summer months (0.2% of total volume per day; Hickey & Banas, 2003). Nevertheless, based on moorings and water samples collected by the Washington Department of Ecology (1999–2000), strong gradients in temperature and salinity exist (Ruesink et al., 2003). At the mouth, salinity and temperature show oceanic signals, but salinity drops below 25 in parts of the bay over 20 km from the mouth.

We focused our sampling on three sites in the middle of the bay's southern arm: Nahcotta on the west (NA: 46°30′ N, 124°01′ W), Nemah on the east (NE: 46°31′ N, 123°57′ W), and a site in between (LI: 46°31′ N, 123°59′ W) at the north end of Long Island (Fig. 1). At each site we identified six distinct habitat types: unstructured mud/sand (bare), eelgrass bed, longline aquaculture, hand-picked ground aquaculture, dredged ground aquaculture, and oyster hummocks. The aquaculture habitats occurred in distinct beds (~1–4 ha) at each site. In addition, we collected samples from two habitats (unstructured mud/sand, hummock) at river-influenced sites at South Bend (SB: 46°40′ N, 123°49′ W), Palix River (PR: 46°37′ N, 123°55′ W), and the Willapa Bay National Wildlife Refuge (WR: 46°25′ N, 123°56′ W), and from two habitats (eelgrass bed, hand-picked ground culture) near the mouth of the bay at Stackpole (ST: 46°36′ N, 124°01′ W) (Fig. 1). The full suite of habitat types was not available at the extreme ends of the estuarine gradient.

Field sampling

We visited sites during morning low tides on July 30–31, 2003. In each habitat (bed), sediment was collected from four randomly selected points spaced up to 100 m apart, with each point consisting of three cores (1.27 cm diameter, 5 cm depth). These three cores were homogenized and stored on ice until processed within 12 h. Simultaneously, we recorded reduction-oxidation potential (mV) in surface sediments using a redox meter fitted with a platinum electrode and filled with AgCl solution (Thermo

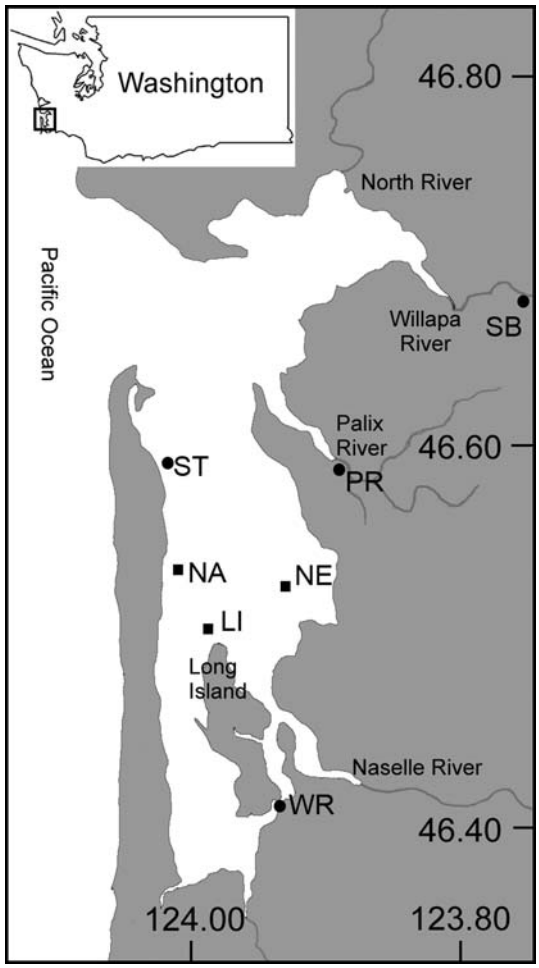

Fig. 1 Map of Willapa Bay showing mid-bay sites (squares), where sediments were collected from six habitats, and additional sites spanning the estuary gradient (circles), where fewer habitat types were present. Two-letter codes as in *Methods... Study sites*

Orion Company, Beverly, MA, USA). Although the redox measurement was not entirely coincident with the full core depth, we used it as an indicator of aerobic vs. anaerobic conditions. For all analyses, the four samples in each bed were averaged, because they were not replicates of habitat type, and the alternative approach of carrying out nested analyses provides little additional power (Gotelli & Ellison, 2004).

Bacterial counts

Processing to determine cell densities of all bacteria (aerobic and anaerobic) followed Porter & Feig (1980). Bacteria were collected from sediment samples by shaking 5 g damp sediment in 45 ml cold-sterilized (0.22 μm filtered) seawater at room temperature. The bacterial supernatant was diluted to 1/100 strength, and we added 1 ml formalin and 1 ml solution of 4′, 6-Diamidino-2-Phenylindole (DAPI; 1 μg/ml) to 5 ml of the bacterial extract. After 10 min, 1 or 2 ml of the stained solution was filtered through a black polycarbonate disk filter (0.22 μm). Prokaryotic assemblages were enumerated using digital epiflourescent microscopy (10 pictures per disk).

Sole-source carbon use (SSCU)

Biolog^{TM} Ecology plates containing 31 unique carbon sources and a redox dye (tetrazolium violet) were plated using the same 1:100 dilutions of sediment core extracts used for prokaryotic density enumerations. Carbon sources in the plate are common in soils: β-Methyl-D-Glucoside, D-Galactonic Acid γ-Lactone, L-Arginine, Pyruvic Acid Methyl Ester, D-Xylose, D-Galacturonic Acid, L-Asparagine, Tween 40, i-Erythritol, 2-Hydroxy Benzoic Acid, L-Phenylalanine, Tween 80, D-Mannitol, 4-Hydroxy Benzoic Acid, L-Serine, α-Cyclodextrin, N-Acetyl-D-Glucosamine, γ-Hydroxybutyric Acid, L-Threonine, Glycogen, D-Glucosaminic Acid, Itaconic Acid, Glycyl-L-Glutamic Acid, D-Cellobiose, Glucose-1-Phosphate, α-Ketobutyric Acid, Phenylethylamine, α-D-Lactose, D,L-α-Glycerol Phosphate, D-Malic Acid, and Putrescine. The dye changes color when it is reduced by bacterial respiration, providing an indicator of the use of different carbon sources by the assemblage of aerobic microbes (Garland & Mills, 1991). We added 1.5 ml of bacterial solution to each well and recorded color development over 10 days (at 0, 4, 8, 12, 16, 20, 36, 48, 60, 72, 84, 96, 120, 144, 168, and 240 h). Five plates had filtered seawater added as a control. Color change in the dye leveled off after 5 days. Overall aerobic SSCU was calculated from the sum of well color development across all 31 carbon sources.

Sediment characteristics

Subsamples ($\sim$30 g dry weight) of sediment were wet-sieved through 63 μm mesh to separate silt (<63 μm) from sand (>63 μm). Fractions were dried

at 65°C for at least 72 h prior to weighing. Separate samples (~5 g dry weight) were dried at 65°C to constant weight and ashed at 550°C to constant weight to determine organic content based on loss-on-ignition.

Statistical analyses

Our sampling was designed as a randomized complete block with three blocks (Nemah, Long Island, Nahcotta; random factor) and six habitat types (fixed factor) within each block. Habitats were not replicated within blocks. We carried out randomized block analyses of variance (ANOVA) on bacterial cell density and overall aerobic SSCU (well color development), followed by Tukey's HSD (highly significant difference) if habitat proved significant. We also tested similarly for habitat differences in sediment characteristics: organic content, silt:sand, and redox potential.

Diversity of SSCU by aerobic microbes among habitat types was examined with principal components analysis (PCA), applied to the correlation matrix for color development of the 31 carbon sources (Primer 5.0, Plymouth, UK). We carried out Analysis of Similarity (ANOSIM), which compares the observed Bray–Curtis similarity matrix to randomizations (in this case 9999), to determine which habitat types were more different than expected by chance.

Samples from all seven sites, which included the ocean and freshwater ends of the estuarine gradient (Fig. 1), were included in correlations among microbes and sediment characteristics. Specifically, we tested for correlations between organic content and silt:sand, redox and silt:sand, prokaryotic cell density and organic content, and overall aerobic SSCU and organic content. Altogether, we studied the relationship of sediments and microbes across the entire estuarine gradient in Willapa Bay, but the six habitat types were compared only in mid-bay.

Results

Overall SSCU of aerobic microbes in the sediments of Willapa Bay varied by habitat type (Fig. 2A). Overall SSCU was calculated as the sum of well color development on Biolog plates, which were incubated under aerobic conditions. In ANOVA, blocked by site, habitat was a significant factor (SS = 193.7, $F_{5,10} = 4.1$, $P = 0.03$). Post-hoc tests showed that hand-picked aquaculture beds had lower activity of aerobic bacteria than did eelgrass beds or bare areas, and all habitats with oysters on-bottom showed this trend of lower SSCU (Fig. 2A). Interestingly, prokaryotic cell density in sediments, which included both anaerobic and aerobic types, was not correlated with activity at these sites in the middle of the bay ($r = 0.05$, $P = 0.85$, $N = 18$), and there was no significant difference in cell density among habitat types, due to substantial within-habitat variation (Fig. 2B; SS = 9.78×10^{20}, $F_{5,10} = 3.0$, $P = 0.50$).

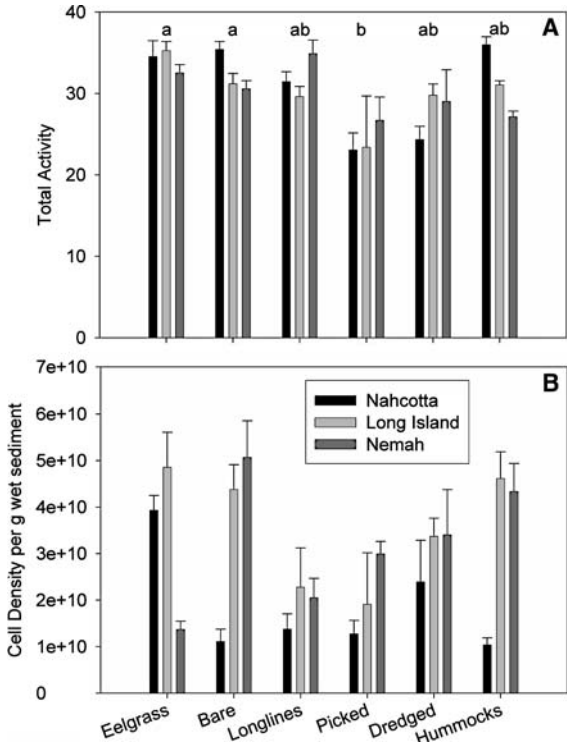

Fig. 2 (**A**) Overall aerobic sole-source carbon use (SSCU) and (**B**) abundance (aerobic and anaerobic) of microbes collected from six habitat types in three sites in Willapa Bay. Habitats are arranged from vegetated to unstructured to four types with oysters. Longlines hold oysters off-bottom; hand-picked and dredged oysters occur on-bottom; hummocks occur where oysters recruit naturally. Bars represent standard error of four nested samples. In **A**, habitats that share letters cannot be distinguished statistically

Diversity of aerobic SSCU, like overall SSCU, varied among habitat types (Fig. 3). The first principal component (PC1) explained 36.1% of the variation in degradation of 31 carbon sources

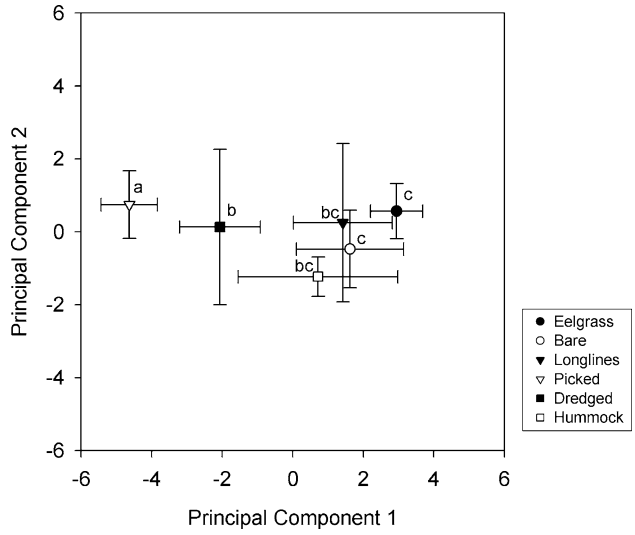

Fig. 3 Principal components analysis of aerobic sole-source carbon use (SSCU) by sediment microbes in Willapa Bay. Points represent the average (+/– standard error) of the first two principal components for each habitat across three sites. Points that share letters cannot be distinguished statistically based on ANOSIM of the correlation matrix

(normalized), the second principal component explained 15.3% of the variation, and additional principal components each explained <10%. Most (28 of 31) carbon sources had positive loadings of 0.1–0.25 on PC1, which mainly represented well color development across all carbon sources. ANOSIM showed significant variation in SSCU among habitat types (Global $R = 0.23$, $P = 0.032$), and in pairwise tests, eelgrass beds were significantly different from dredged and hand-picked aquaculture, hand-picked were also different from all other habitat types, and dredged and bare beds were different (Fig. 3).

At the three sites in the middle of Willapa Bay, sediment characteristics were similar throughout (Table 1): 1–6% organic content, and silt:sand ratios of 0.1–1. In fact, in randomized block ANOVA, habitat type was never a significant predictor variable of sediment characteristics: organic content (SSQ = 11.6, $F_{5,10} = 0.91$, $P = 0.51$); log (silt:sand) (SSQ = 0.8, $F_{5,10} = 0.79$, $P = 0.58$); redox potential (SSQ = 46141, $F_{5,10} = 1.57$, $P = 0.25$).

Although sediment characteristics were similar throughout the mid-bay, regardless of habitat type (Table 1), they varied strongly across the estuarine gradient (Fig. 4). At this scale, many sediment characteristics covaried among sample locations. Organic content and silt:sand ratio were strongly related, with siltier sediments having higher organic content (Fig. 4A; $r = 0.84$, $P < 0.001$, $N = 26$). Up-estuary sites (South Bend, Palix River, National Wildlife Refuge) were particularly silty (silt:sand = 9) and high in organic content (9%). High silt:sand was also related to reduced redox potential (Fig. 4B;

Table 1 Environmental variables measured at each study site in mid-bay

Habitat types	Nahcotta	Long Island	Nemah
Eelgrass	3.69 (0.19)	5.90 (1.11)	1.50 (0.07)
	0.44 (0.07)	0.32 (0.02)	0.05 (0.005)
	–116 (10)	53 (21)	63 (7)
Bare sediment	3.09 (0.19)	5.34 (2.11)	5.65 (0.75)
	0.16 (0.007)	0.22 (0.007)	1.04 (0.16)
	–67 (13)	25 (8)	–32 (17)
Longline off-bottom	0.94 (0.32)	5.07 (0.61)	3.17 (0.27)
	0.06 (0.02)	0.31 (0.02)	0.27 (0.01)
	na	82 (15)	–71 (15)
Hand-picked on-bottom	0.77 (0.20)	2.20 (1.05)	3.97 (0.22)
	0.04 (0.006)	0.32 (0.08)	0.24 (0.04)
	na	68	30 (21)
Dredged on-bottom	0.93 (0.05)	4.10 (0.70)	4.30 (0.31)
	0.03 (0.002)	0.29 (0.04)	0.35 (0.03)
	72 (14)	–58 (43)	–103 (18)
Hummocks	5.42 (0.67)	3.50 (0.32)	4.01 (0.76)
	0.98 (0.23)	0.38 (0.08)	0.43 (0.07)
	–183 (16)	–84 (21)	–115 (9)

Each cell in the table reports organic content (%), silt:sand, and oxidation-reduction potential (mV). Values are averages of four nested samples from each bed (standard error in parentheses); only averages were used in analyses. na = not available

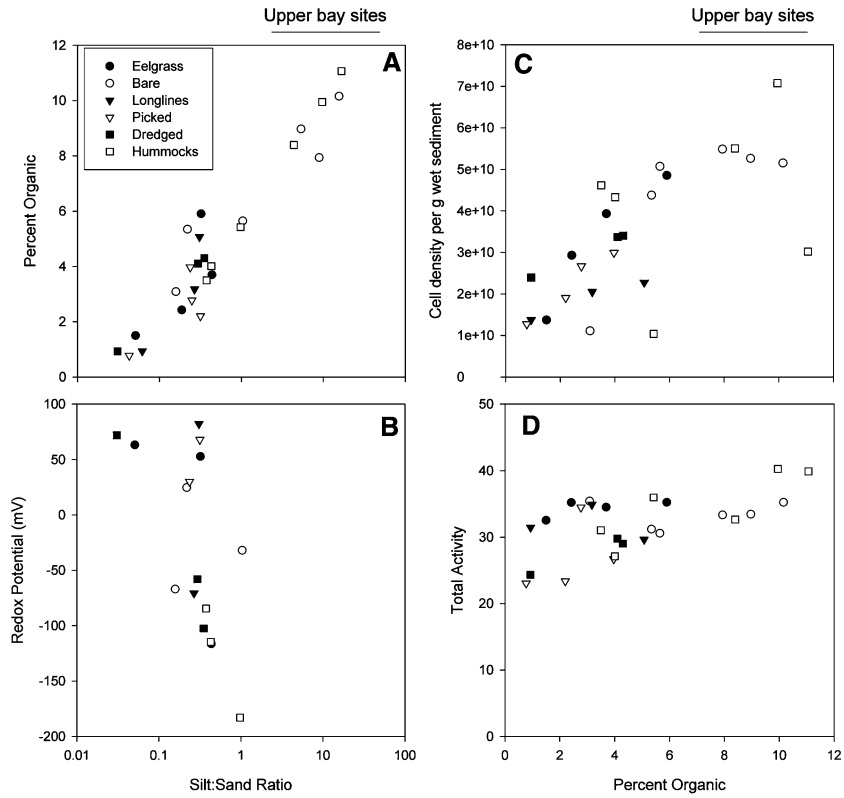

Fig. 4 Environmental characteristics and microbial assemblages in Willapa Bay at sites spanning the entire estuarine gradient. (**A**) Silt:sand and organic content of sediments. (**B**) Silt:sand and redox potential of sediments. (**C**) Organic content and microbial cell density in sediments. (**D**) Organic content and overall aerobic sole-source carbon use (SSCU). Individual points are an average of four nested samples. Correlations in text

$r = –0.54$, $P = 0.03$, $N = 16$), although sampling was sparse at lower-salinity locations. Sediment characteristics were strongly associated with microbial abundance: silty, organic, low-redox sites had higher bacterial cell counts (Fig. 4C; $r = 0.70$, $P < 0.0001$, $N = 26$). Overall SSCU by aerobic bacteria was also positively related to organic content (Fig. 4D; $r = 0.60$, $P = 0.001$, $N = 26$), but the trend was much less dramatic: whereas cell density varied 7-fold across sites, overall aerobic SSCU varied by <50%.

Discussion

Biogenic habitat-forming species such as oysters and eelgrass have been predicted to modify conditions for other species, including the microbial assemblage (Kaspar et al., 1985; Cifuentes et al., 2000; Newell et al., 2002). Our summer sampling in Willapa Bay showed that aerobic microbial metabolism varied by habitat, based on carbon-source utilization (Figs. 2, 3). This variation arose despite the fact that we found no consistent variation in sediment characteristics across habitat types (Table 1, Fig. 4).

Two aspects of these results require some explanation: first, why was there no consistent variation in sediment characteristics across habitat types? Given the strong impacts of biogenic species observed in other published studies, we were initially puzzled by the lack of any consistent difference in sediment properties between habitats with and without oysters in Willapa Bay. However, in this observational study, we could not control for differences other than biogenic habitat that might exist across beds, for instance, small-scale hydrodynamics. In addition, the accumulation of small particles within eelgrass beds (Madsen et al., 2001) may mimic the biodeposits of oysters (Bayne & Hawkins, 1992) thus producing a similar effect on the sediments, albeit via different mechanisms. Finally, harvest activities remove fine particles due to disturbance of sediment as oysters are picked up or dredged. Cultured oysters (as opposed to hummocks) often have such short tenures on tideflats that there may be no time for biodeposits to accumulate. Taken together, these phenomena could cause eelgrass and oyster hummock habitats to have similar sediment conditions, but oyster aquaculture habitats to be variable, depending on how recently or

frequently oysters were harvested (Table 1). Thus, a habitat effect on sediments would be difficult to detect. The effects of oysters and eelgrass on sediment properties should be explored experimentally to control for other variables.

The second aspect of the results in need of explanation is why patterns in aerobic SSCU would differ from bacterial cell abundance and sediment characteristics. In our study, overall aerobic SSCU was generally higher in vegetated habitat than in oyster beds, even though abundance (aerobic and anaerobic) did not differ (Fig. 2). This lack of correlation may be due to the fact that our measures of metabolism only account for the aerobic component of the active bacterial fraction. For instance, samples with lower aerobic SSCU may contain more anaerobic bacteria contributing to total cell density. Interestingly, Braker et al. (2001), in their genetic study of subtidal sediment microbes around Washington state, found that aerobic and anaerobic types were well-mixed, even though distinct metabolic activities occurred with sediment depth. We can infer that a decline in aerobic SSCU might not require a decline in cell number, but only in activity if conditions were less favorable. Although we found no significant variation in reduction-oxidation potential among habitats, the probe may have been too coarse to reveal high-oxygen conditions around eelgrass root tips (Jensen et al., 2005), and all surface sediments would be expected to be re-oxygenated regularly in this macrotidal estuary. Aerobic SSCU may also be decoupled from sediment properties due to spatial variation in the biopolymeric fraction (carbohydrates, proteins, and lipids) (Pusceddu et al., 1999). We measured only the quantity, not the quality of organic material available for microbial metabolism. Finally, we compared aerobic metabolism during a single season, and, although warm summer temperatures would be expected to encourage decomposition in general, large additions of labile organic detritus and biodeposits may disproportionately favor anaerobes, whose metabolism we did not measure.

In general, carbon-source utilization has several limitations for describing functional diversity of microbes. There is no one-to-one relationship between carbon-source utilization and taxonomy: several genetic types may be able to metabolize a single carbon source, and some genetic types that are abundant in the natural environment do not appear in any of the Biolog wells (Ruger & Krambeck, 1994; Smalla et al., 1998). Since Biolog plates were developed for terrestrial systems, some marine carbon sources may also be different. Although we were interested in carbon-source utilization because oysters and eelgrass supply different materials for detrital food webs, there were no obvious carbon sources used in one habitat but not another: The first principal component reflected color development in all wells of the Biolog plates. Thus, the statistical analyses of overall aerobic SSCU (Fig. 2A) and diversity of aerobic SSCU (Fig. 3) gave similar results, with the latter allowing somewhat more distinction among habitat types. Similarly, aerobic SSCU differed for microbes from native eelgrass (*Z. marina*) and non-native eelgrass (*Zostera japonica*) primarily due to PC1, which reflected overall well color, even though cell densities were equivalent (Hahn, 2003).

Suspension-feeding bivalves have been reported to alter sediment characteristics of marine systems in a variety of ways. At high biodeposition rates, bivalves can form fine-grained sediments with high organic content and low oxygen content (Rodhouse & Roden, 1987). Interestingly, their effects on nutrient concentrations in the sediment are not necessarily straightforward: although biodeposition may lead to elevated nitrogen (Kautsky & Evans, 1987; Deslous-Paoli et al., 1992; Hatcher et al., 1994), it may also create a combination of aerobic and anaerobic layers facilitating nitrification–denitrification processes that ultimately release nitrogen gas (Newell et al., 2002). It would be useful to measure nutrient fluxes to determine if the ratios of aerobic to anaerobic microbes in large beds of oysters actually increase denitrification. Overall, however, our results are consistent with lower biogeochemical consequences of shellfish aquaculture, such as intensive off-bottom oyster (Rajendran et al., 1999) and mussel aquaculture (Danovaro et al., 2004), compared to finfish farms.

Based on sites throughout Willapa Bay, both sediment properties and microbial assemblages vary across the estuarine gradient from more oceanic to riverine sites (Fig. 4). The explanation rests on simple physics, because small particles tend to settle out in low-energy environments. Correlations among grain size, organic content, and redox potential of the sort we observed are also typical of marine sediments (Gray, 1981). Microbes are commonly associated

with organic content (Blum et al., 2004; Koster et al., 2005), as our data demonstrate. The shift in aerobic SSCU was much smaller than cell density across the estuarine gradient, and a likely explanation is that many of the cells in up-estuary sites represented anaerobic taxa (Fig. 4C, D).

Variation in aerobic microbial metabolism across habitat types was statistically significant, although less variable than across the estuarine gradient (Fig. 2A vs. Fig. 4D). Interestingly, our data revealed that SSCU varied by habitat despite no consistent variation in sediment characteristics, so aerobic metabolism did not vary through the anticipated pathway of changes in sediment. In general, aerobic metabolism was higher in eelgrass beds than in aquaculture beds with intertidal on-bottom oysters (Fig. 2A, Fig. 3). What remains to be determined is if the microbial response intensifies or mitigates aquaculture's impact on estuarine biogeochemistry. Careful management of shellfish beds may be necessary to achieve desired ecosystem functioning in terms of nutrient cycling (Newell, 2004). However, in the case of Willapa Bay, these results must be considered in the context of strong and correlated gradients in bacterial communities and biogeochemical functions along the estuary as a whole (Fig. 4).

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